

Data Evaluation Record on the Toxicity of Fluopyram (AE C656948) + Tebuconazole (HWG 1608) SC 400 G on Seedling Emergence and Growth of Terrestrial Plants
EPA MRID Number 47567614

Data Requirement: EPA DP Barcode: D386298
EPA Guideline: OPPTS 850.4100 (Tier I)

Test material: FLU+TBZ SC200+200A G **Purity:** 17.9% w/w FLU, 17.8% w/w TBZ
Common name
Chemical name: Fluopyram (AE C656948) and Tebuconazole (HWG 1608)

Primary Reviewer: Stephen Carey, Biologist
EPA/OCSPP/OPP/EFED/ERB6

Signature: 
Date: 7/29/11

Secondary Reviewer(s):
{EPA/OECD/PMRA}

Date:

Reference/Submission No.: {.....}

EPA PC Code 080302/128997

CITATION: Gosch, H. & Nguyen, D.H. 2007. Non-target Terrestrial Plants: An Evaluation of the Effects of AE C656948 & Tebuconazole SC 400 (200 + 200) g/L in the Seedling Emergence and Growth Test (Tier I). Unpublished study performed by Bayer CropScience AG, Frankfurt am Main, Germany. Laboratory Project Number: SE07/04. Document No. M-293230-01-2. Report ID. EBGMP025. Study sponsored by Bayer CropScience AG, Frankfurt am Main, Germany. Study completed October 5, 2007.

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Executive Summary:

The purpose of this specific study was to evaluate the effect of Fluopyram + tebuconazole SC 400 (200 + 200) g/L on the seedling emergence and seedling growth of dicotyledonous (cucumber (*Cucumis sativus*), oilseed rape (*Brassica napus*), soybean (*Glycine max*), sugar beet (*Beta vulgaris*), sunflower (*Helianthus annuus* L.), tomato (*Lycopersicon esculentum*) and buckwheat (*Fagopyrum esculentum*)) and monocotyledonous (oat (*Avena sativa*), onion (*Allium cepa*) and corn (*Zea mays*)) plant families over a 14 day period. Ten species of terrestrial non-target plants (3 monocots and 7 dicots) were treated at an application rate of 1.0 L product (Fluopyram + tebuconazole SC 400 (200 + 200) g/L) / ha. All seeds were planted one day before application and test duration was 14 days after 70% emergence of the seedlings in the controls for each species. Spray treatments were applied once, at test initiation, with a sprayer set at the nominal spray volume of 200 liters/ha. Control pots were sprayed with deionized water. Four replicates with five seeds per pot for each species were tested. All pots were individually contained in saucers and retained on benches within a greenhouse.

Plants were assessed for emergence, survival and rated for phytotoxicity on days 7 and 14. At study termination (day 14), biomass endpoint determinations were performed for plant dry weights. % Inhibition for biomass was reduced in cucumber, oilseed rape, soybean, sugar beet, sunflower, tomato, buckwheat and ryegrass by 41.1%, 28%, 38.8%, 36% 4.8%, 20% 24.7% and 16% respectively. The EC25 is < 1 L product/ha for cucumber, oilseed rape, soybean and sugar beet, which were inhibited 25% or more; further Tier II testing for those species is needed to establish an EC25 and NOAEC. Inhibition of all other parameters was less than 25% when compared to control. The most sensitive monocot species was ryegrass, with 16% reduction in biomass when compared to control; the EC25 and NOAEC were determined to be >1 and ≥ 1 L product/ha, respectively. The most sensitive dicot species was cucumber with a 41% effect in biomass when compared to control. For some dicot species including the most sensitive dicot (cucumber) the EC25 and NOAEC were determined to be < 1 L product/ha.

Buckwheat, corn and ryegrass were the only species to exhibit no phytotoxic effects. Slight to severe effects were observed in a few instances and effects included chlorosis and stunting.

The study is scientifically sound but does not satisfy EPA guideline requirement for a Tier I Seedling Emergence toxicity study. The study is classified as SUPPLEMENTAL.

Results Synopsis**Monocot**

EC₂₅/IC₂₅: >1 L product/ha

NOAEC: ≥ 1 L product/ha

Most sensitive monocot: Ryegrass

Most sensitive parameter: Biomass

Dicot

EC₂₅/IC₂₅: <1 L product/ha

NOAEC: < 1 L product/ha

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Most sensitive dicot: Cucumber

Most sensitive parameter: Biomass

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I) Material and Methods

Guideline Followed:

OECD 208 (July 2006): seedling emergence and growth test (Tier 1)

Compliance:

None. This report does not meet the requirements for EPA FIFRA GLP, 40 CFR Part 160.

A. Materials

1. Test material:

Fluopyram (AE C656948) + tebuconazole (HWG 1608) SC 200 + 200

Specification No:

102000016375

Batch No:

2007-002120

Purity:

Nominal: 200 g fluopyram/L + 200 g tebuconazole/L
Analyzed: 201 g fluopyram /L (17.9 % w/w) and 200 g tebuconazole /L (17.8% w/w)

Visual appearance:

white suspension

Density:

1.123 g/mL

Testing rate of product:

1 L product/ha

2. Vehicle and/or positive control

Deionized water

3. Test organism:

7 dicotyledonous plant species from 7 different families:
cucumber (*Cucumis sativus*),
oilseed rape (*Brassica napus*),
soybean (*Glycine max*),
sugar beet (*Beta vulgaris*),
sunflower (*Helianthus annuus* L.),
tomato (*Lycopersicon esculentum*)
buckwheat (*Fagopyrum esculentum*);
3 monocotyledonous plant species from 2 families:
oat (*Avena sativa*),
onion (*Allium cepa*)
corn (*Zea mays*)

Source:

Seeds used on the study had not been treated with pesticides or repellents prior to test initiation. Seeds were supplied from commercial sources via Bayer CropScience AG, Horticulture, H 872, 65926 Frankfurt am Main. Routine germination tests were carried out on the seeds to ensure their viability. Seeds were stored in plastic box in refrigerator.

Plant growth stage at application::

Seeds

Acclimatisation:

Greenhouse conditions

4. Environmental conditions:

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Temperature:	Regulated at 23 ± 8°C day, 18 ± 8°C night
Photoperiod:	16:8 hours light/dark
Light intensity:	Natural daylight supplemented by artificial lighting to provide the required photoperiod. >15000 lux lamps turn off, >50000 lux shading closes.

B. Study design and methods

1. Experimental dates: 2007-07-04 to 2007-07-30
2. Experimental treatment:

The day before application the seeds were introduced manually in the soil. They were covered by about 2 - 5 mm of soil. Each pot contained 5 seeds. 4 pots per treatment group were used. All seeds were planted one day before application and test duration was 14 days after 70% emergence of the seedlings in the controls for each species.

After sowing the pots were be top watered. Later, bottom watering was performed via saucers standing below each pot. Water was given in the saucer according to the need of the plants in order to have an optimal water supply for plant growth. Sowing and application occurred during the same day.

The soil used is a mixture of 90% sandy-silt loam and 10% washed sand sterilized with 120 degrees vapor for about 30 minutes, fertilized with 2.4 g Blaukorn per liter and sieved to 2 mm. Soil composition: 39.7% sand, 43.1% silt and 17.2% clay; pH 7.3; organic carbon content 0.81%; lime content 2.0%.

The spray solution was applied to the soil surface. The blank control spray solution was deionized water. The test item was dissolved in deionized water and was applied once with 200 L/ha using an spray chamber equipped with an overhead nozzle (Teejet 8001EVS), with nozzle height set at 35 cm above the sprayed surface. The spray chamber volume was calibrated by weighing the amount of water applied to a known surface area.

3. Observations:

Analytical verification: No analytical verification of the stock solution was conducted.

Endpoints:

Emergence (germination):	Daily checks were made to identify the date when 70% of the seedlings emerge in the control for each species. Numbers of plants were counted after 7 and 14 days.
Survival:	Numbers of plants that survived after application were recorded at the final assessment.
Phytotoxicity:	Visual phytotoxicity ratings were recorded (e.g. chlorosis, necrosis, and stunting, abnormal growth) 7 and 14 days after the emergence of 70% of seeds in the controls.
Growth stages:	Growth stages were recorded at the final assessment according to BBCH Monograph- Growth stages.
Biomass:	The dry weights were determined at the final assessment (day 14 after 70% emergence in the untreated control).

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Statistical methods

Statistical analysis of biomass data was carried out using the Pairwise Mann-Whitney-U-test (one sided).

II) Results and Discussion

Findings

A summary of effects of foliar application Fluopyram + tebuconazole SC 400 (200 + 200) g/L at a application rate of 1 L product/ha on survival, phytotoxicity, and biomass (weight) is given in the following table:

Table 1: Effects on emergence, survival, phytotoxicity, and biomass (weight) of 10 plant species after a foliar application of Fluopyram & Tebuconazol SC 200+200 in a seedling emergence test					
	Emergence * % inhibition	Survival * % inhibition	Phytotoxicity **	Dry Weight *** % inhibition	Growth stages ****
Cucumber	-25	0	B-C af	41.1	12 (12)
Oilseed rape	-20	0	B-C af	28.0	14 (14)
Soybean	5.3	0	B-C af	38.8	12 (12)
Sugar beet	0	0	A af	36.0	14 (14)
Sunflower	0	0	A f	4.8	14 (14)
Tomato	-5.3	0	A f	20.0	12-14 (10-14)
Buckwheat	5.3	0	0	24.7	12 (12)
Corn	0	0	0	-9.6	14 (14)
Oat	0	0	A f	-6.1	21 (21)
Ryegrass	0	0	0	16.0	21 (21)

* An increase in dry weight is given as negative values.
 ** Phytotoxicity was recorded at each assessment time with the following a rating system:
 0 no injury or effect
 A slight symptom(s) throughout the whole plant or more moderate symptom(s) on a small area, i.e. one leaf
 B moderate symptom(s) throughout the whole plant or severe symptom(s) on a limited area, i.e. one-two leaves
 C severe symptom(s) throughout the whole plant with younger or newly developed leaves growing normally
 D total plant symptom(s) with the plant showing poor vigour.
 E moribund
 a = chlorosis (yellowing of green shoot tissue)
 b = necrosis (brown shoot tissue)
 c = bleaching (shoot tissue without any pigmentation)
 d = wilting (loss of turgor of shoot tissue)
 e = leaf deformation (leaf curl, abnormal leaf shape)
 f = stunting (plant height reduced with shorter inter-node lengths)
 *** inhibition is expressed on a per plant basis. An increase in dry weight is given as negative values.
 **** growth stages of the control in parentheses

Observations

There was no adverse effect of 1.0 L/ha AE C656948 + Tebuconazole SC 200 + 200 g/L on the survival of

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the ten species tested. Visible phytotoxicity (stunting and/or chlorosis) was observed in cucumber, oilseed rape, soybean, sugar beet, sunflower, tomato and oat. Germination was increased in cucumber, oilseed rape and tomato by 25%, 20% and 5.3% respectively. Germination was inhibited in soybean and buckwheat by 5.3% both. Biomass was reduced in cucumber, oilseed rape, soybean, sugar beet, sunflower, tomato, buckwheat and ryegrass by 41.1%, 28%, 38.8%, 36% 4.8%, 20% 24.7% and 16% respectively. Biomass was increased in corn and oat by 9.6% and 6.1% respectively. Differences were significant for cucumber and soybean at the 95% confidence limits. The author reported none of these differences reached or exceeded 50% to trigger further testing.

III) Conclusion of study author:

A nominal product application rate of 1.0 L/ha Fluopyram + tebuconazole SC 400 (200 + 200) g/L showed no adverse effect (above 50% as compared to the control) for all the tested plant species in the seedling emergence test.

The LR₅₀ and ER₅₀ can be estimated to be > 1 L product/ha, respectively.

EPA Reviewer's Conclusion:

The test was terminated on Day 14; EPA guideline 850.4100 requires 21 days.

Three monocots were tested; EPA guideline 850.4100 requires four monocots.

Did not include EPA FIFRA Good Laboratory Practices standards, since not subjected to GLP.

Biomass was reduced in cucumber, oilseed rape, soybean, sugar beet, tomato, buckwheat and ryegrass by 41.1%, 28%, 38.8%, 36%, 20% 24.7% and 16% respectively. The EPA reviewer conducted t-tests to establish the EC25s and NOAECs since the author did not calculate them for the ten species (see Appendix I). When the limit concentration was statistically significant different from control or inhibited 25% or more, an EC25 and NOAEC could not be determined and are determined to be below the lowest test concentration tested. For some species (including the most sensitive dicot) the EC25 and NOAEC were determined to be below the lowest test concentration tested which impacts the acceptability of the study. A multiple-concentration study (tier II) may be necessary to establish EC25s and NOAECs for those species to satisfy EPA guidelines.

The EPA Reviewer agrees with the conclusion of the study author that the foliar application foliar application Fluopyram + tebuconazole SC 400 (200 + 200) g/L at a application rate of 1 L product/ha had no relevant (>50%) effect on emergence, survival, phytotoxicity, biomass (weight), and growth stages for the 10 tested species under the test conditions described in OECD 208. However, EPA guideline OPPTS 850.4100 requires an EC25 and NOAEC. Because biomass was reduced more than 25% in several species; the EC25 and NOAEC are both < 1 L product/ha that trigger further Tier II testing to determine the EC25 and NOAEC.

Monocot:

EC₅₀ : > 1 L product/ha nominal
 EC₂₅ : > 1 L product/ha nominal

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NOAEC: ≥ 1 L product/ha nominal
Most sensitive monocot: Ryegrass (based on 16% reduction)
Most sensitive parameter: Biomass

Dicot:

EC₅₀ : > 1 L product/ha nominal
EC₂₅ : < 1 L product/ha nominal
NOAEC: < 1 L product/ha nominal
Most sensitive monocot: Cucumber (based on 41% reduction)
Most sensitive parameter: Biomass

REFERENCES

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Appendix I. EPA Reviewer's Statistical Results

Statistical Method(s): Any species exhibiting an inhibition of 5% in survival, dry weight or plant height at the control or 1 L product/ha treatment level relative to the negative control was statistically analyzed using a t-test for two samples assuming equal variance. If inhibitions were <5% at the control level or treatment level relative to the negative control, the reviewer visually determined that no significant differences were present. Either species inhibited $\geq 25\%$ or with a p value of 0.05 or smaller are determined to be < 1 L product/ha. All analyses were conducted using the nominal application rate of 1 L product/ha.

Table A-1. Effect of 1 L product/ha of Fluopyram + tebuconazole SC 400 (200 + 200) g/L on biomass at 14 days after application

Species	Treatment (L product/ha)	Mean biomass out of 10 seeds	% reduction from negative control ¹	EC25 (lbs a.i./A)	P value ^{2,3}	NOEC (lbs a.i./A)
Cucumber	control (n=4)	0.545	--	<1	n/c	<1
	1 (n=4)	0.321	41			
Oilseed rape	control (n=4)	0.321	--	<1	n/c	<1
	1 (n=4)	0.231	28			
Soybean	control (n=4)	0.39	--	<1	n/c	<1
	1 (n=4)	0.238	38.8			
Sugarbeet	control (n=4)	0.192	--	<1	n/c	<1
	1 (n=4)	0.123	36			
Sunflower	control (n=4)	0.279	--	>1	n/a	≥ 1
	1 (n=4)	0.266	4.8			
Tomato	control (n=4)	0.088	--	>1	0.24	≥ 1
	1 (n=4)	0.07	20			
Buckwheat	control (n=4)	0.263	--	>1	0.25	≥ 1
	1 (n=4)	0.198	24.7			
Corn	control (n=4)	0.615	--	>1	n/a	≥ 1
	1 (n=4)	0.674	-9.6			
Oat	control (n=4)	0.166	--	>1	n/a	≥ 1
	1 (n=4)	0.176	-6.1			
Ryegrass	control (n=4)	0.032	--	>1	0.182	≥ 1
	1 (n=4)	0.027	16			

¹ Negative values indicate an increase relative to the negative control.

² A t-test is required when any endpoint exhibits $\geq 5\%$ inhibition at the maximum dose when compared to negative control. For any endpoint with less than 5% inhibition, a t-test is not required.

³ With a p value of 0.05 or smaller indicates a significant effect at the maximum dose and a tier II study with this species may be required.

n/a – not applicable

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n/c – not calculated

Ryegrass

0.037 0.023
 0.033 0.033
 0.025 0.025
 0.032 0.026

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	Variable 2
Mean	0.03175	0.02675
Variance	2.49167E-05	1.89167E-05
Observations	4	4
Pooled Variance	2.19167E-05	
Hypothesized Mean Difference	0	
df	6	
t Stat	1.510420081	
P(T<=t) one-tail	0.090840714	
t Critical one-tail	1.943180274	
P(T<=t) two-tail	0.181681428	
t Critical two-tail	2.446911846	

Buckwheat

0.133 0.133
 0.337 0.202
 0.281 0.25
 0.302 0.208

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	Variable 2
Mean	0.26325	0.19825
Variance	0.008073583	0.00234825
Observations	4	4
Pooled Variance	0.005210917	
Hypothesized Mean Difference	0	
df	6	
t Stat	1.273418898	
P(T<=t) one-tail	0.12498847	
t Critical one-tail	1.943180274	
P(T<=t) two-tail	0.249976941	
t Critical two-tail	2.446911846	

Tomato

0.051 0.066
 0.099 0.061
 0.103 0.087
 0.098 0.066

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	Variable 2
Mean	0.08775	0.07

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Variance	0.000604917	0.000134
Observations	4	4
Pooled Variance	0.000369458	
Hypothesized Mean Difference	0	
df	6	
t Stat	1.305962245	
P(T<=t) one-tail	0.119698561	
t Critical one-tail	1.943180274	
P(T<=t) two-tail	0.239397123	
t Critical two-tail	2.446911846	
